

Effects of a Single Ketamine Dose on the Potentiated Startle Response in the Wistar-Kyoto Rat Model of Affective Dysfunction

Alena Lemeshova, 2026

Haney Haidari, 2027

Cherish Zhao, 2028

Background:

The management of treatment-resistant disorders, such as depression and anxiety is one of the most widely researched topics in neuropharmacology. A specific interest has recently been on ketamine, a non-competitive NMDA receptor antagonist, which produces a swift therapeutic response with long term effects (lasting up to 7 days) after a single administration (Gass et al. 2019; Murrough et al. 2013; Yang et al. 2016).

However, the exact mechanism of ketamine's antidepressant effects remains unknown. Furthermore, due to standard poor translational preclinical animal models, the specific Wistar Kyoto rats are used (WKY; Will et al. 2003). This is due to WKY rats being selectively bred to exhibit depressive and anxiety-like symptoms comparable to patients (McAuley et al. 2009). Lastly, only female subjects were used in this project, as they are underrepresented and due to evidence suggesting that estrogen's interactions with ketamine enhances its effectiveness even at lower doses. (Lehmann et al. 1999; Wright et al. 2016)

Hence, this project utilizes the WKY strain as a model to measure the animals' behavior in an acoustic startle paradigm, assessing the degree of response to a loud unexpected stimulus - as they show maladaptive startle responses, synonymous to human patients with depression (McAuley et al. 2009; Vaidyanathan et al. 2013). We hypothesize that ketamine treatment will normalize this maladaptive behavior both immediately (24 hours after) and longer term (7 days after), and will correlate with the degree of oxidation in the targeted brain areas (Lemeshova 2024; Réus et al. 2015). Lastly, we also hypothesize that ketamine's different efficacies will depend on the phase of the hormonal estrus cycle on the day of administration (Wright et al. 2016).

Methodology, Experimental Procedure, and Timelines

Animals: 44 WKY experimental rats and 12 Wistar control rats were 21 days old upon arrival. After handling and acclimation, at the age of 47 days (the start of their young adult phase), the WKY rats were divided into three experimental groups based on dosage and one control group.

Ketamine Treatment: A single ketamine dose of 5 mg/kg, 10 mg/kg, or 15 mg/kg was intraperitoneally injected (Bærentzen et al. 2024; Manduca et al. 2020; McDonnell et al. 2021). All rats in the experimental group received the treatment at 50 days and were tested 24 hours and 7 days later, with the control group receiving comparable doses of saline.

Behavioral and Molecular Experimentation: Before ketamine treatment, all 47-day-old rats were acclimated in the SR-LAB Startle Response System to remove confounding effects of stress on the expression of the relevant neural markers this study uses, including parvalbumin, after testing (Palmer and Printz, 1999). Both immediate and delayed behavioral trials were conducted using protocols previously used in the Honeycutt laboratory. Upon placement of the rats in the startle box, every experimental session included 30 trials of subsequent 100-millisecond sound cues of 95-, 105-, or 115-dB white noise in a randomized order with 30- to 45-second intervals in between. Average startle response and maximum startle response were assessed as voltage, reported in millivolts.

After the final behavioral testing, brain collection and immunohistochemistry analysis were conducted to examine activation patterns in the basolateral amygdala, hippocampus, and prefrontal cortex, as these areas are associated with anxiety (Campbell and McQueen, 2004; Sharp, 2017; Hare and Duman, 2020; Ghasemi et al., 2022). Staining was done for parvalbumin, a protein correlated to neuronal inhibition and stress-related disorders, to measure ketamine's impact on inhibitory behaviors (Gildawie et al., 2020). Oxidative stress was also assessed through analysis of 8-hydroxy-2'-deoxyguanosine (8-OHdG), which is a well-established neural marker for oxidative damage, investigating ketamine's potential neuroprotective effects (Gürler et al., 2014; Xiao et al., 2017).

Results

It was concluded that there was a decreasing trend in startle response with increasing ketamine dose. Specifically, the 15 mg/kg ketamine dose significantly suppressed the startle response, potentially indicating an anxiolytic or antidepressant effect at higher doses.

Faculty Mentor: Jennifer Honeycutt

Funded by the Life Sciences Summer Research Fellowship

References

1. Ahmed GK, Elserogy YM, Elfadl GMA, Ghada Abdelsalam K, Ali MA. 2023. Antidepressant and anti-suicidal effects of ketamine in treatment-resistant depression associated with psychiatric and personality comorbidities: A double-blind randomized trial. *Journal of Affective Disorders*. 325:127–134. doi:<https://doi.org/10.1016/j.jad.2023.01.005>.
2. Alshammari, T. K. (2020). The Ketamine Antidepressant Story: New Insights. *Molecules*, 25(23), 5777. <https://doi.org/10.3390/molecules25235777>
3. Campbell, S., & Macqueen, G. (2004). The role of the hippocampus in the pathophysiology of major depression. *Journal of psychiatry & neuroscience : JPN*, 29(6), 417–426.
4. Gass N, Becker R, Reinwald J, Cosa-Linan A, Sack M, Weber-Fahr W, Vollmayr B, Sartorius A. 2019. Differences between ketamine's short-term and long-term effects on brain circuitry in depression. *Translational Psychiatry*. 9(1):172. doi:<https://doi.org/10.1038/s41398-019-0506-6>.
5. Ghasemi, M., Navidhamidi, M., Rezaei, F. *et al.* Anxiety and hippocampal neuronal activity: Relationship and potential mechanisms. *Cogn Affect Behav Neurosci* 22, 431–449 (2022). <https://doi.org/10.3758/s13415-021-00973-y>
6. Gildawie KR, Honeycutt JA, Brenhouse HC. 2020. Region-specific effects of maternal separation on perineuronal net and parvalbumin-expressing interneuron formation in male and female rats. *Neuroscience*. 428:23–37. doi:10.1016/j.neuroscience.2019.12.010. <https://doi.org/10.1016/j.neuroscience.2019.12.010>.
7. Gürler HŞ, Bilgici B, Akar AK, Tomak L, Bedir A. 2014. Increased DNA oxidation (8-OHdG) and protein oxidation (AOPP) by low level electromagnetic field (2.45 GHz) in rat brain and protective effect of garlic. *International Journal of Radiation Biology*. 90(10):892–896. doi:10.3109/09553002.2014.922717. <https://doi.org/10.3109/09553002.2014.922717>.
8. Hare, B. D., & Duman, R. S. (2020). Prefrontal cortex circuits in depression and anxiety: contribution of discrete neuronal populations and target regions. *Molecular psychiatry*, 25(11), 2742–2758. <https://doi.org/10.1038/s41380-020-0685-9>
9. Lehmann J. 1999. Sex differences in the acoustic startle response and prepulse inhibition in Wistar rats. *Behavioural Brain Research*. 104(1-2):113–117. doi:[https://doi.org/10.1016/s0166-4328\(99\)00058-3](https://doi.org/10.1016/s0166-4328(99)00058-3).
10. Maeng LY, Cover KK, Landau AJ, Milad MR, Lebron-Milad K. 2015. Protocol for studying extinction of conditioned fear in naturally cycling female rats. *Journal of Visualized Experiments*.(96). doi:10.3791/52202. <https://doi.org/10.3791/52202>.

11. McAuley JD, Stewart AL, Webber ES, Cromwell HC, Servatius RJ, Pang KCH. 2009. Wistar–Kyoto rats as an animal model of anxiety vulnerability: Support for a hypervigilance hypothesis. *Behavioural Brain Research*. 204(1):162–168. doi:<https://doi.org/10.1016/j.bbr.2009.05.036>.
12. Murrough JW, Iosifescu DV, Chang LC, Al Jurdi RK, Green CE, Perez AM, Iqbal S, Pillemer S, Foulkes A, Shah A, et al. 2013. Antidepressant Efficacy of Ketamine in Treatment-Resistant Major Depression: A Two-Site Randomized Controlled Trial. *American Journal of Psychiatry*. 170(10):1134–1142. doi:<https://doi.org/10.1176/appi.ajp.2013.13030392>.
13. Sharp B. M. (2017). Basolateral amygdala and stress-induced hyperexcitability affect motivated behaviors and addiction. *Translational psychiatry*, 7(8), e1194. <https://doi.org/10.1038/tp.2017.161>
14. Vaidyanathan U, Welo EJ, Malone SM, Burwell SJ, Iacono WG. 2013. The effects of recurrent episodes of depression on startle responses. *Psychophysiology*. 51(1):103–109. doi:<https://doi.org/10.1111/psyp.12152>.
15. Will CC, Aird F, Redei EE. 2003. Selectively bred Wistar–Kyoto rats: an animal model of depression and hyper-responsiveness to antidepressants. *Molecular Psychiatry*. 8(11):925–932. doi:<https://doi.org/10.1038/sj.mp.4001345>.
16. Wright KN, Strong CE, Addonizio MN, Brownstein NC, Kabbaj M. 2016. Reinforcing properties of an intermittent, low dose of ketamine in rats: effects of sex and cycle. *Psychopharmacology*. 234(3):393–401. doi:10.1007/s00213-016-4470-z. <https://doi.org/10.1007/s00213-016-4470-z>.
17. Xiao M, Zhong H, Xia L, Tao Y, Yin H. 2017. Pathophysiology of mitochondrial lipid oxidation: Role of 4-hydroxynonenal (4-HNE) and other bioactive lipids in mitochondria. *Free Radical Biology and Medicine*. 111:316–327. doi:10.1016/j.freeradbiomed.2017.04.363. <https://doi.org/10.1016/j.freeradbiomed.2017.04.363>.
18. Yang J, Wang N, Yang C, Shi J, Yu H, Hashimoto K. 2015. Serum Interleukin-6 Is a Predictive Biomarker for Ketamine's Antidepressant Effect in Treatment-Resistant Patients With Major Depression. *Biological Psychiatry*. 77(3):e19–e20. doi:<https://doi.org/10.1016/j.biopsych.2014.06.021>.